

WHAT IS CLAIMED IS:

1. A vascular endothelial growth factor (VEGF) dimer consisting of a first and a second monomer each comprising at least amino acids 11 to 116 of SEQ ID NO: 1, or comprising an amino acid sequence having at least about 90% sequence identity with amino acids 11 to 116 of SEQ ID NO: 1, and retaining a cysteine (Cys) at or corresponding to position 116 of SEQ ID NO: 1 (Cys-116), wherein Cys-116 of each monomer is disulfide-bonded to an additional extraneous Cys.
2. The VEGF dimer of claim 1 wherein in at least one of said first and second monomers said additional Cys is part of a peptide of 2-5 amino acids.
3. The VEGF dimer of claim 2 wherein said peptide is glutathione.
4. The VEGF dimer of claim 3 wherein each monomer is disulfide bonded, through a Cys residue, to a glutathione moiety.
5. The VEGF dimer of claim 1 wherein said first and second monomers comprise amino acids 4 to 116 of SEQ ID NO: 1.
6. The VEGF dimer of claim 1 wherein said first and second monomers comprise amino acids 1 to 120 of SEQ ID NO: 1.
7. The VEGF dimer of claim 1 wherein said first and second monomers comprise amino acids 1 to 121 of SEQ ID NO: 1.
8. The VEGF dimer of claim 1 wherein said first and second monomers comprise amino acids 5 to 120 of SEQ ID NO: 1.
9. The VEGF dimer of claim 1 wherein the length of at least one of said first and second monomers does not exceed 121 amino acids.
10. The VEGF dimer of claim 9 wherein the length of each of said first and second monomers does not exceed 121 amino acids.
11. The VEGF dimer of claim 10 wherein the length of each of said first and second monomers is between 110 and 121 amino acids.
12. The VEGF dimer of claim 1 wherein both of said first and second monomers are glycosylated.
13. The VEGF dimer of claim 1 wherein at least one of said first and second monomers is unglycosylated.
14. A composition comprising a vascular endothelial growth factor (VEGF) dimer consisting of a first and a second monomer each comprising at least amino acids 11 to 116 of SEQ ID NO: 1, or comprising an amino acid sequence having at least about 90% sequence identity with amino acids 11 to 116 of SEQ ID NO: 1, and retaining a cysteine (Cys) at or corresponding to position 116 of SEQ ID NO: 1 (Cys-116), wherein Cys-116 of each monomer is disulfide bonded to an additional extraneous Cys, in admixture with a pharmaceutically acceptable vehicle.

15. The composition of claim 14 wherein in at least one of said first and second monomers said additional Cys is part of a peptide of 2-5 amino acids.

16. The composition of claim 15 wherein said peptide is glutathione.

17. The composition of claim 16 wherein each monomer is disulfide bonded, through a Cys residue, to a glutathione moiety.

18. The composition of claim 14 wherein said first and second monomers comprise amino acids 4 to 116 of SEQ ID NO: 1.

19. The composition of claim 14 wherein said first and second monomers comprise amino acids 1 to 120 of SEQ ID NO: 1.

20. The composition of claim 14 wherein said first and second monomers comprise amino acids 1 to 121 of SEQ ID NO: 1.

21. The composition of claim 14 wherein said first and second monomers comprise amino acids 5 to 120 of SEQ ID NO: 1.

22. The composition of claim 14 wherein both of said first and second monomers are glycosylated.

23. The composition of claim 14 wherein at least one of said first and second monomers is unglycosylated.

24. The composition of claim 14 wherein each of said first and second monomers is unglycosylated.

25. The composition of claim 24 wherein said first and second monomers additionally comprise an N-terminal methionine group.

26. The composition of claim 24 essentially free of a VEGF dimer in which the cysteines at or corresponding to position 116 of each monomer are connected with an interchain disulfide bond.

27. The composition of claim 24 essentially free of a VEGF dimer in which the cysteines at or corresponding to position 116 of each monomer are unpaired.

28. A composition of matter comprising at least two vascular endothelial growth factor (VEGF) dimers, each formed by a first and a second monomer, selected from the group consisting of:

(a) a dimer in which each monomer comprises amino acids 11 to 116 of SEQ ID NO: 1, or comprises an amino acid sequence having at least about 90% sequence identity with amino acids 11 to 116 of SEQ ID NO: 1, and retaining a cysteine (Cys) at or corresponding to position 116 of SEQ ID NO: 1 (Cys-116), and Cys-116 of each monomer is disulfide-bonded to an additional extraneous Cys;

(b) a dimer in which each monomer comprises amino acids 11 to 116 of SEQ ID NO: 1, or comprises an amino acid sequence having at least about 90% sequence identity with amino acids 11 to 116 of SEQ ID NO: 1, and retaining a cysteine (Cys) at or corresponding to position 116 of SEQ ID NO: 1 (Cys-116), and the Cys-116's of the two monomers are connected with an interchain disulfide bond; and

(c) a dimer in which each monomer comprises amino acids 11 to 116 of SEQ ID NO: 1, or comprises an amino acid sequence having at least about 90% sequence identity with amino acids 11 to 116 of SEQ ID NO: 1, and retaining a cysteine (Cys) at or corresponding to position 116 of SEQ ID NO: 1 (Cys-116), and Cys-116 of one or both monomers is unpaired;

5 wherein in each of said dimers (a) - (c) said first and second monomers are independently glycosylated or unglycosylated.

29. The composition of matter of claim 28 wherein in at least one of dimers (a) - (c), each monomer comprises amino acids 1 to 120 of SEQ ID NO: 1.

10 30. The composition of matter of claim 28 wherein in at least one of dimers (a) - (c), each monomer comprises amino acids 1 to 121 of SEQ ID NO: 1.

31. The composition of matter of claim 28 comprising, as its main component, a dimer in which each monomer comprises amino acids 1 to 120 of SEQ ID NO: 1, and Cys-116 of each monomer is disulfide bonded to an additional Cys.

15 32. The composition of matter of claim 31, wherein said main component constitutes at least about 75% of the amount of VEGF dimers present.

33. The composition of matter of claim 32, wherein said main component constitutes at least about 85% of the amount of VEGF dimers present.

34. The composition of matter of claim 33, wherein said main component constitutes at least about 95% of the amount of the VEGF dimers present.

20 35. A process for providing a composition of matter comprising VEGF polypeptides, wherein said VEGF polypeptides comprise at least two vascular endothelial growth factor (VEGF) dimers, each formed by a first and a second monomer, selected from the group consisting of:

(a) a dimer in which each monomer comprises amino acids 11 to 116 of SEQ ID NO: 1, or comprises an amino acid sequence having at least about 90% sequence identity with amino acids 11 to 116 of SEQ ID NO: 1, and retaining a cysteine (Cys) at a position corresponding to position 116 of SEQ ID NO: 1 (Cys-116), and Cys-116 of each monomer is disulfide bonded to an additional extraneous Cys;

25 (b) a dimer in which each monomer comprises amino acids 11 to 116 of SEQ ID NO: 1, or comprises an amino acid sequence having at least about 90% sequence identity with amino acids 11 to 116 of SEQ ID NO: 1, and retaining a cysteine (Cys) at a position corresponding to position 116 of SEQ ID NO: 1 (Cys-116), and the Cys-116's of the two monomers are connected with an interchain disulfide bond; and

30 (c) a dimer in which each monomer comprises amino acids 11 to 116 of SEQ ID NO: 1, or comprises an amino acid sequence having at least about 90% sequence identity with amino acids 11 to 116 of SEQ ID NO: 1, and retaining a cysteine at a position corresponding to position 116 of SEQ ID NO: 1 (Cys-116), and the Cys-116 of one or both monomers is unpaired;

35 wherein in each of said dimers (a) - (c) said first and second monomers may be independently glycosylated or unglycosylated, said process comprising:

providing transformed host cells comprising a species of exogenously added DNA encoding a polypeptide of SEQ ID NO: 1, or encoding a polypeptide the amino acid sequence of which has at least about 90% sequence identity with amino acids 11 to 116 of SEQ ID NO: 1, present in an operable expression vector,

5 culturing said host cells under conditions suitable for expression of said DNA and the synthesis of said VEGF polypeptides, and

recovering said VEGF polypeptides.

36. The process of claim 35 wherein in at least one of said dimers (a) - (c) each monomer comprises amino acids 1 to 120 of SEQ ID NO: 1.

10 37. The process of claim 35 wherein in at least one of said dimers (a) - (c) each monomer comprises amino acids 1 to 121 of SEQ ID NO: 1.

38. The process of claim 35 wherein at least about 95% of said VEGF polypeptides is unglycosylated.

15 39. The process of claim 35 wherein at least about 95% of said VEGF polypeptides is devoid of an N-terminal methionine residue.

40. The process of claim 35 wherein said dimer (a) comprises amino acids 1 to 121 of SEQ ID NO: 1.

41. The process of claim 40 wherein said dimer (a) constitutes at least about 85% of said VEGF polypeptides.

20 42. The process of claim 35 additionally comprising the step of purifying said polypeptides.

43. The process of claim 35 wherein said transformed host cells are bacterial cells.

44. The process of claim 43 wherein said bacterial cells are *E. coli* cells.

25 45. The process of claim 43 wherein the exogenously added DNA encodes a polypeptide of SEQ ID NO: 1 extended by a Met-(AA)_n- sequence at the amino terminus (N-terminus), wherein Met stands for methionine, n is 1-7, and AA represents identical or different amino acids, where at least one of the AA amino acids, or a combination of two or more of the AA amino acids, is capable of retarding proteolytic degradation of the mature N-terminus of the VEGF polypeptide by the bacterial host cell.

46. The process of claim 45 wherein n is 1.

30 47. The process of claim 46 wherein AA represents an amino acid selected from the group consisting of lysine (Lys) and arginine (Arg) residues.

48. The process of claim 47 wherein AA represents a lysine (Lys) residue.

49. The process of claim 45 further comprising the step of purifying said VEGF polypeptides.

35 50. The process of claim 49 further comprising the removal of the N-terminal Met(AA)_n- sequence following at least partial purification.

51. The process of claim 50 wherein removal is performed by enzymatic digestion.

52. The process of claim 43 further comprising the step of refolding said VEGF polypeptides.

53. The process of claim 52 wherein refolding is performed in a refolding buffer comprising cysteine and cystine in amounts and in a ratio to each other sufficient to produce the desired mixture of VEGF dimers.

54. A process for producing a vascular endothelial growth factor (VEGF) dimer composed of two VEGF monomers, in which each monomer comprises amino acids 11 to 116 of SEQ ID NO: 1, or comprises an amino acid sequence having at least about 90% sequence identity with amino acids 11 to 116 of SEQ ID NO: 1, and retaining a cysteine (Cys) at a position corresponding to position 116 of SEQ ID NO: 1 (Cys-116), where Cys-116 of each monomer is disulfide bonded to an additional extraneous Cys, comprising the steps of:

(a) providing transformed bacterial host cells comprising a species of exogenously added DNA encoding a polypeptide of SEQ ID NO: 1 extended by a Met-(AA)_n- sequence at the amino terminus (N-terminus), wherein Met stands for methionine, n is 1-7, and AA represents identical or different amino acids, where at least one of the AA amino acids, or a combination of two or more of the AA amino acids, is capable of blocking the retarding proteolytic degradation of the mature N-terminus of the VEGF polypeptides by the bacterial host cell, present in an operable expression vector,

(b) culturing said bacterial host cells under conditions suitable for expression of said DNA and the synthesis of said N-terminally-extended VEGF monomers, and

(c) recovering said VEGF dimer.

55. The process of claim 54 wherein n is 1.

56. The process of claim 55 wherein AA represents an amino acid selected from the group consisting of lysine (Lys) and arginine (Arg) residues.

57. The process of claim 56 wherein AA represents a lysine (Lys) residue.

58. The process of claim 54 further comprising the step of purifying said VEGF dimer.

59. The process of claim 58 further comprising the removal of the N-terminal Met(AA)_n- sequence following at least partial purification.

60. The process of claim 59 wherein removal is performed by enzymatic digestion.

61. The process of claim 54 additionally comprising the step of refolding said VEGF dimer.

62. The process of claim 56 additionally comprising the step of refolding said VEGF dimer.

63. The process of claim 59 additionally comprising the step of refolding said VEGF dimer.

64. The process of claim 63 wherein refolding is performed in a refolding buffer comprising cysteine and cystine.

65. A process for blocking the removal of one or more amino acids from the mature amino terminus (N-terminus) of a polypeptide during production in a bacterial host cell, comprising transforming said bacterial host cell with DNA encoding said polypeptide extended at its N-terminus by a Met-(AA)_n sequence, wherein Met stands for methionine, n is 1-7, and AA represents identical or different amino acids, where at least one of the AA amino acids, or a combination of two or more of the AA amino acids, is capable of retarding proteolytic degradation of the mature N-terminus of the polypeptide by the bacterial host cell.

66. The process of claim 65 wherein said polypeptide is longer than 100 amino acids.

67. The process of claim 66 wherein said polypeptide is a VEGF molecule.

68. A method of inducing angiogenesis or vascular remodeling, comprising administering to a patient in need an effective amount of the composition of claim 35.

69. A method for the treatment of peripheral arterial disease, comprising administering to a patient in need an effective amount of the composition of claim 35.

70. A method for the treatment of coronary artery disease, comprising administering to a patient in need an effective amount of the composition of claim 35.

71. A method for the treatment of essential hypertension, comprising administering to a patient in need an effective amount of the composition of claim 35.

72. A method for the treatment of microvascular angiopathy, comprising administering to a patient in need an effective amount of the composition of claim 35.

73. A method for the treatment of polycystic kidney disease, comprising administering to a patient in need an effective amount of the composition of claim 35.

74. A method for the repair of vascular endothelial cell layers, comprising administering to a patient in need an effective amount of the composition of claim 35.